

-28-

CLAIMS

1. Protein derived from a *Streptococcus pneumoniae* PBP2x, characterized in that it consists of a concatenation of the fragments corresponding respectively 5 to the amino acids located between positions 74 to 90, 186 to 199, 218 to 228 and 257-750, with reference to the sequence of the PBP2x protein of the strain R6 (SWISSPROT P14677 or GENBANK 18266817), each one of said fragments being preceded by a peptide fragment of 1 to 7 amino 10 acids.

2. Protein according to Claim 1, characterized in that said peptide fragment comprises amino acids of said *Streptococcus pneumoniae* PBP2x protein located between positions -1 to -7, relative to the residues at positions 74, 186, 218 and 257, and/or between 15 positions +1 to +7, relative to the residues at positions 90, 199 and 228, as defined in Claim 1.

3. Protein according to Claim 1 or Claim 2, characterized in that said peptide fragment comprises 20 amino acids chosen from alanine (A), serine (S), glycine (G) and threonine (T).

4. Protein according to any one of Claims 1 to 3, characterized in that it is derived from a  $\beta$ -lactam-resistant strain of *S. pneumoniae*.

25 5. Protein according to any one of Claims 1 to 3, characterized in that it has the sequence SEQ ID No. 1.

30 6. Protein according to any one of Claims 1 to 5, characterized in that it comprises a substitution of at least one methionine residue with a seleno-methionine residue.

7. Protein according to any one of Claims 1

REPLACED BY  
101734407

-29-

to 6, characterized in that it is associated with a ligand.

8. Protein according to any one of Claims 1 to 7, characterized in that it is in the form of a 5 crystal.

9. Peptide, characterized in that it consists of a fragment of at least 7 amino acids of the mini-PBP2x protein, according to any one of Claims 1 to 6, which peptide includes at least one residue chosen from those 10 located at positions 74, 90, 186, 199, 218, 228 and 257 as defined in Claim 1.

10. Antibodies, characterized in that they are directed against a peptide according to Claim 9.

11. Isolated nucleic acid molecule, characterized in that it is selected from the group consisting of the sequences encoding a mini-PBP2x according to any one of Claims 1 to 6 and the sequences complementary to the preceding sequences, which are sense or antisense.

12. Pair of primers, characterized in that it 20 has the sequence SEQ ID Nos. 2-3.

13. Probes and primers, characterized in that they comprise a sequence of approximately 10 to 30 nucleotides corresponding to that located at the junction of the peptide fragments of 1 to 7 amino acids and 25 the fragments of PBP2x as defined in Claim 1.

14. Primers according to Claim 13, characterized in that they have a sequence selected from the group consisting of the sequences SEQ ID Nos. 4 to 9.

15. Recombinant vector, characterized in that 30 it comprises an insert selected from the group consisting of the nucleic acid molecules encoding a mini-PBP2x according to Claim 11.

REPLACED BY  
ART 34 AMEND.

-30-

16. Expression vector according to Claim 15, characterized in that it consists of a prokaryotic vector.

5 17. Cells transformed with a recombinant vector according to either one of Claims 15 and 16.

18. Cells according to Claim 17, characterized in that they are prokaryotic cells.

19. Use of a mini-PBP2x according to any one of Claims 1 to 8, for screening antibiotics.

10 20. Method for screening antibiotics, characterized in that it comprises at least the following steps:

a<sub>1</sub>) bringing a mini-PBP2x according to any one of Claims 1 to 7 into contact with a test substance,

15 b<sub>1</sub>) detecting, by any suitable means, the binding of said test molecule with the mini-PBP2x and/or the inhibition of the activity of said mini-PBP2x resulting from this binding, and

20 c<sub>1</sub>) selecting and identifying the active substances capable of binding to the mini-PBP2x and/or of inhibiting the activity of said mini-PBP2x, which can be used as antibiotics.

25 21. Method for identifying antibiotics, characterized in that it comprises at least the following steps:

a<sub>2</sub>) preparing crystals from a mini-PBP2x according to any one of Claims 1 to 7,

30 b<sub>2</sub>) determining the three-dimensional structure of said mini-PBP2x from the crystal obtained in a<sub>2</sub>), and

c<sub>2</sub>) identifying active substances capable of binding to the mini-PBP2x and/or of inhibiting the

REPLACED BY  
ART 34 AMDT

-31-

activity of said mini-PBP2x, which can be used as antibiotics.

22. Screening kit for implementing the method according to Claim 20 or Claim 21, characterized in that 5 it includes at least one protein, one peptide, one antibody, one vector, one cell, one probe or one primer, according to any one of Claims 1 to 18.

REPLACED BY  
ART 34 AMDT